Isolation of mononuclear cells from human bone marrow aspirates by density gradient centrifugation

Contents

1. Reagent and instrument requirements

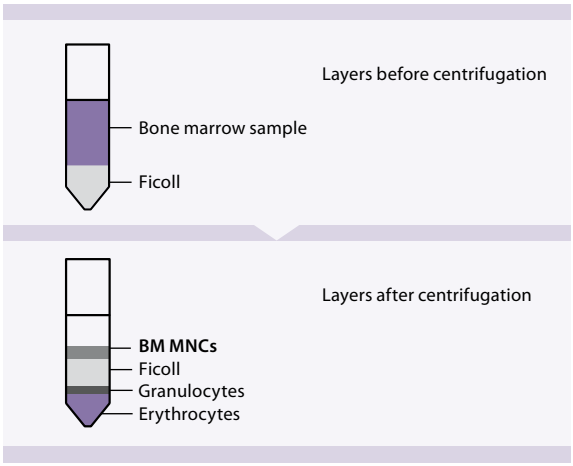
- Buffer: Prepare a solution containing sterile phosphate-buffered saline (PBS), pH 7.2, and 2 mM EDTA. Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD).

- Filter (pore size 100 µm) to remove bone fragments and cell clumps.
- 15 mL Ficoll-Paque™ (ρ = 1.077 g/mL).

2. Protocol

2.1 Schematic figure of a density gradient centrifugation



2.2 Preparation of human bone marrow mononuclear cells (BM MNCs)

▲ Use fresh bone marrow only. Avoid freezing and thawing of bone marrow cells and perform all of the following steps under sterile conditions in a laminar flow hood.

1. Collect bone marrow from the upper iliac crest or the sternum by using an aspiration needle.
2. Dilute aspirated human bone marrow at a ratio of 7:1 with buffer, e.g., dilute 30 mL of bone marrow with 5 mL of buffer to a final volume of 35 mL.
3. Pass cells through a 100 µm filter to remove bone fragments and cell clumps.

   ▲ **Note:** Wet filter with buffer before use.
4. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque in a 50 mL conical tube.
5. Centrifuge at 445×g for 35 minutes at 20 °C in a swinging bucket rotor without brake.
6. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
7. Carefully transfer the BM MNCs at the interphase to a new 50 mL conical tube.
8. Wash cells by adding up to 40 mL of buffer, mix gently and centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant completely.
9. For removal of platelets, resuspend the cell pellet in 50 mL of buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove the supernatant completely.

   ▲ **Note:** This step will increase the purity of the target cells in the subsequent MACS® Cell Separation.
10. Resuspend cell pellet in an appropriate amount of buffer for downstream applications. For magnetic labeling see MACS Cell Separation Reagents data sheets.

    ▲ **Note:** BM MNCs may be stored in the refrigerator overnight in PBS containing 0.5% BSA or autologous serum. Do not store cells longer than one day in the refrigerator. Wash at least once before proceeding to magnetic labeling and resuspend cells in an appropriate buffer. For details see MACS Cell Separation Reagents data sheets.

All protocols and data sheets are available at **www.miltenyibiotec.com.**

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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